[illegible]02DV2 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	O'Brien et al.	Group Art Unit 1812
Appl. No.	:	08/484,594	
Filed	:	June 7, 1995	
For	:	USE OF PROSAPOSIN AND NEUROTROPHIC PEPTIDES DERIVED THEREFROM (AS AMENDED)	
Examiner	:	R. Hayes, Ph.D.	

DECLARATION OF JOHN S. O'BRIEN, M.D. UNDER RULE 132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, John S. O'Brien, M.D., declare and state:

1. I am an inventor of the above-identified application and am familiar with the application, claims and file history thereof.

2. I received my Master's Degree in 1958 and my M.D. degree in 1960, both from Creighton University, Omaha, Nebraska. From 1962-1964, I was an instructor in the Departments of Pathology and Medicine at the University of Southern California (USC). From 1964-1968, I was Chief of the Section of Molecular Pathology at the USC School of Medicine, and from 1964-1967 I was an Assistant Professor in the same department. From 1968-1970, i was an Associate Professor in the Department of Neurosciences at the University of

California, San Diego (UCSD) School of Medicine. I have been a Professor in the Department of Neurosciences at the UCSD School of Medicine for the last 26 years and during that time have held various positions within the UCSD Department of Neurosciences including that of Director of the Division of Neurometabolic Disorders and Chairman of the Department of Neurosciences.

3. I have received numerous honors and awards including the Foster Elting Bennett Lecturer Award in 1976, the Jacob Javits Neurosciences Investigator Award in 1984 and the Chancellor's Associates Merit Award for Excellence in Research from UCSD in 1985. i belong to numerous professional societies, including the American Federation for Clinical Research, the American Association for Pathologists and the American Society for Neurochemistry. I have authored more than 200 publications, the majority of which were published in prestigious peer-reviewed journals.

4. For the last 31 years, my research has focused on the structure and function of lipids and lipid-associated enzymes in the central and peripheral nervous system and their role in lipid storage diseases. In particular, for the last 7 years, I have been involved in research on prosaposin and saposins derived therefrom.

5. I understand that in the Office Action dated July 22, 1996, the Examiner has expressed skepticism concerning the ability of prosaposin and peptides derived therefrom to affect the myelination process. Example 2 of the specification evidences increased myelination in mouse cerebellar explants. The cell population of explants comprises a heterogeneous cell environment that mimics the *in vivo* environment. Thus, it is predictive of *in vivo* results.

Appl. No. : 08/484,594
Filed : June 7, 1995

6. Enclosed herewith is data from my laboratory showing the prevention of cell death (demyelination) in immortalized Schwann cells in culture (Exhibit A). The saposin C-derived peptide 769P (the 22-mer peptide disclosed in the present application) reduced Schwann cell death in a dose-dependent manner. Exhibit B shows the effect of the 22-mer peptide on the incorporation of ^{35}S into sulfolipids in primary myelin-containing Schwann cells. Sulfolipids are exclusively incorporated into myelin. Cells were incubated in low sulfate media, then ^{35}S-methionine was added for 48 hours, Cells were then assayed for ^{35}S incorporation into lipids by standard methods. As shown in the graph, the peptide significantly increased the amount of myelin-specific lipids produced in primary Schwann cells.

7. Fujita et al. (*Hum. Mol. Genet.*, 5:711-725, 1996) (Exhibit C) inactivated the prosaposin gene in mice, resulting in mutant mice homozygous for the prosaposin mutation. These mice exhibited two clinical phenotypes-neonatally fatal and later-onset. The latter developed rapidly progressive neurological signs around 20 days and died by 35-38 days. Severe hypomyelination was noted at 30 days, underscoring the importance of prosaposin in normal neural myelination (See Figure 3).

8. I also understand that the Examiner questioned whether the *in vitro* and *ex vivo* data disclosed in the application would correlate with *in vivo* efficacy. Enclosed herewith are three references demonstrating the *in vivo* neurotrophic activity of prosaposin and peptides derived therefrom containing the active neurotrophic fragment located within amino acids 8-29 of SEQ ID NO: 3. Sano et al. (*Biochem. Biophys. Res. Commun.*, 204:994-1000, 1994) (Exhibit D) and Kotani et al. (*J. Neurochem.*, 66:2197-2200, 1996) (Exhibit E) demonstrate that

infusion of prosaposin and saposin C-derived 18-mer peptide, respectively, into the lateral ventricle of gerbils after 3 min of forebrain ischemia protected against both ischemia-induced learning disability and hippocampal CA1 neuronal loss (Figs. 1 and 2). Kotani et al. (*J. Neurochem.*, 68:2019-2025, 1996) (Exhibit F) show that prosaposin dramatically increased the number of regenerating nerve fibers after sciatic nerve transection *in vivo* (Fig. 2). Furthermore, prosaposin prevented the atrophy of cholinergic large neurons in the anterior horn (Fig. 4).

9. A saposin C-derived peptide, TX14(A), comprising the active neurotrophic fragment located within amino acids 8-29 of SEQ ID NO: 3 (TXLIDNNATEEILY; X=D-alanine) prevented peripheral neuropathy resulting from diabetes and *in vivo* taxol administration. In diabetes, there is an associated sensory neuropathy in which thermal perception is impaired. Streptozotocin-induced diabetic rats were tested for thermal response latency using a Hargraves thermal testing apparatus. Rats were placed on a surface and laser light was shined on a footpad. The response time was then measured in seconds as the time it takes for the rat to withdraw its paw from the surface. As shown in Exhibit G, diabetic rats had an increased response time compared to healthy control animals due to the diabetes-induced neuropathy. However, in animals treated with 20, 200 or 1000 µg/kg of peptide, this response time was significantly reduced and reached control values in the animals treated with 200 and 1000 µg/kg of peptide. Exhibit H shows the results of a similar experiment performed with taxol-treated rats in which 50 mg/kg of taxol was administered either in the presence or

absence peptide. The rats receiving both taxol and the peptide exhibited a decrease in withdrawal time, indicating an improvement in taxol-mediated neuropathy.

10. Enclosed herewith as Exhibit I are two graphs showing motor (MNCV) and sensory (SNCV) conduction velocities in diabetic and control rats. As shown in the graphs, diabetic rats have greatly impaired sensory and motor neuron conduction velocities compared to control rats. In contrast, diabetic rats administered peptide TX14(A) exhibited a dose-dependent increase in both sensory and motor nerve conduction velocities.

11. The Chung rat model (Kim et al., *Pain*, **50**:355, 1992) is an experimental model of peripheral neuropathy produced by segmental spinal nerve (L5-L6) ligation to produce an allodynic state seven days postoperative in which rats become hypersensitive to pressure on the footpad. The 22-mer peptide (SEQ ID NO: 1) was intrathecally administered to rats. As seen in Exhibit J, the 22-mer peptide reversed the hypersensitivity as determined by the increase in threshold pressure tolerated by the rats. At the highest dose (0.7 μg/rat), the hypersensitivity was reversed within 15-20 minutes and this reversal was sustained for at least three hours.

12. The correlation between *in vitro* and *in vivo* activity of neurotrophic factors is generally well accepted. For example, Yao et al. (*Proc. Natl. Acad. Sci. U.S.A.*, **92**:6190-6194, 1995) showed that insulin-like growth factor 1 (IGF-1) significantly reduced the number and areas of demyelinating lesions *in vivo*, thus correlating *in vivo* activity with the known *in vitro* activity of IGF-1 in promoting the survival of oligodendroglia and the formation of myelin sheaths *in vivo* (Exhibit K).

13. I also understand that the Examiner has expressed the concern that separating an axon from its cell body will invariably result in the degeneration of the separated portion, and asserted that "retarding or halting" neuronal degeneration requires functional regeneration of axons already damaged. The methods of the present invention appear to protect vulnerable neurons around the site of injury which would otherwise degenerate. Prosaposin and its neurotrophic fragments will also stimulate outgrowth of these neuronal cells. In addition, in partial nerve injury and in many demyelination diseases, segmental demyelination occurs in which the axons are substantially intact. The Schwann cells in these segmental areas die unless protection from cell death occurs. Prosaposin and its neurotrophic fragments appear to protect Schwann cells from cell death and also promote remyelination. Thus, the invention clearly retards or halts the degeneration of at least some injured myelinated axons, as explained in more detail in paragraphs 8-10.

14. I am also aware that the Examiner inquired whether any peptide other than the 18-mer disclosed in the specification could cross the blood brain barrier. A 14-mer sequence derived from saposin C containing the active neurotrophic region contained within amino acids 9-29 of SEQ ID NO: 3 was radiolabeled and intravenously injected into the tail vein of rats as described in Example 7 of the specification. At 1 hour, 0.05% of the injected dose was present in whole brain. 80-90% of the radioactivity localized in brain parenchyma and 10-20% was found in capillaries. Thus, the 18-mer is not unique in its ability to cross the blood brain barrier, and Example 7 provides a screening procedure for readily identifying peptides which will do so.

Appl. No. : 08/484,594
Filed : June 7, 1995

15. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 11/15/96

By: John S. O'Brien
John S. O'Brien, M.D.

NSB-3836
111496